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Development of a heart valve model surface for optimization of surface modifications

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ABSTRACT

Current bioprosthetic valve replacements (BPVs) are susceptible to myriad complications, including calcification and thrombosis; however, recent research has explored surface modifications to encourage re-endothelialization of the tissue, preventing unwanted blood–tissue interactions. A bioprosthetic valve surface model (BVSM) was developed to facilitate rapid *in vitro* optimization of surface modification techniques for BPVs. The BVSM was manufactured by photopolymerization of PEGDA and collagen type I and subsequent addition of amine-rich peptide to provide reactive sites for surface modification. This BVSM mimics surface mechanical properties of bioprosthetic valve tissue, as measured by micropipette aspiration. The BVSM successfully mimics the latent toxic effects of glutaraldehyde fixation, as shown through MTT assay results. Amine content, assessed by XPS, was shown to be significantly lower in the BVSM than unfixed tissue. However, incubation of the surface with amine-reactive NHS–PEG–Cy5 revealed even coverage of the BVSM surface, suggesting that there exists sufficient surface reactive groups to anchor surface modifications, and that translation of the modification process to tissue will yield more complete modification of the BPV surface. These results indicate successful construction of a BVSM that mimics essential properties of bioprosthetic valve tissue and its usefulness for rapid *in vitro* optimization of surface modification methods for endothelialization.

Statement of Significance

Current bioprosthetic valve replacements are susceptible to many complications, including calcification and thrombosis; however, recent research has explored surface modifications to encourage the integration of the replacement with the native tissue, which would prevent unwanted blood–tissue interactions. However, methods to analyze and optimize such modifications are limited by the complex surface topography, individual variability, and opacity of native tissue. Thus, we have developed a novel bioprosthetic valve tissue model (BVM) which mimics the important features of the bioprosthetic valve tissue and serves as a platform for rapid optimization and testing of surface modification strategies for tissue valves. Thus, the BVM will provide a needed platform to support rapid improvement of clinically available cardiovascular implants.

1. Introduction

Congenital heart defects affect approximately 3.4 in 1000 people in the U.S. population [1] and include conditions such as bicuspid aortic valve and more severe conditions such as Tetralogy of Fallot and transposition of the great arteries, which require replacement of the defective valve early in life. In fact, 2.3 per 1000 children born will undergo an invasive procedure related to a congenital heart defect within the first year of life [1]. Currently, the two primary choices for valve replacement for pediatric patients are mechanical and bioprosthetic valves [2–5]. Mechanical valves, unfortunately, require lifetime anticoagulation therapy, which is not always ideal for young children [4,5]. Bioprosthetic valves, by contrast, do not require anticoagulation therapy, but are still subject to other complications such as thrombosis, inflammation, and calcification [2,5–8]. Recent efforts to avoid such complications in bioprosthetic valves have focused on chemical treatment of the prosthesis to reduce interactions with the chemical, glutaraldehyde, used to crosslink the tissue [9–11],

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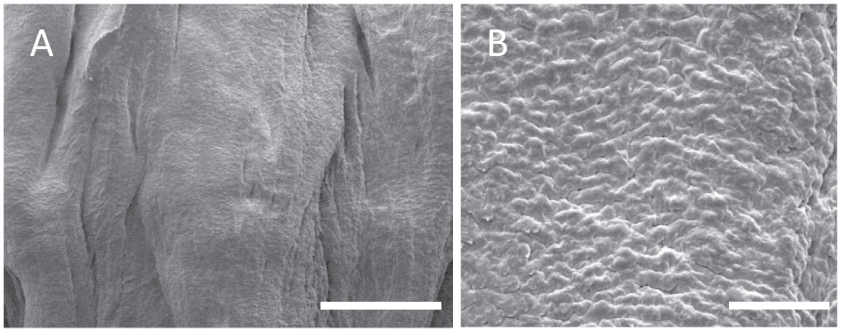

Fig. 1. SEM image of the belly region of a fixed valve leaflet showing the rough folding of the surface at 100× magnification (A, scale bar = 400 μm) and micron-scale surface texturing at 1000× magnification (B, scale bar = 40 μm).

as well as surface modification methods to reduce the interaction of the valve surface with the blood [12–16]. In particular, studies on surface modification have shown promising results, encouraging further development of these technologies to improve their non-thrombogenic and non-immunogenic properties. Additionally, these surface modifications could be designed to be specifically bioactive, attracting and supporting the formation of a protective, native endothelial layer [17–19].

In developing such a non-thrombogenic, non-immunogenic, and endothelial cell-attracting surface coating for heart valves, much optimization must be done to choose the appropriate chemistry, density of bioactive molecules, and other parameters. These surfaces must also be tested by a variety of methods to confirm surface chemistry and mechanics, as well as microscopy to examine cell-surface and blood–surface interactions. Unfortunately, the native heart valve surface has an extraordinarily complex topography (Fig. 1) at multiple length scales and is difficult to image via light microscopy, thus making analysis of the surface markedly more difficult. Furthermore, such optimization using tissues would introduce variability due to differences between individuals from which the tissue was harvested. Thus, we have developed a bioprosthetic valve surface model (BVSM) which mimics the essential characteristics of the surface of bioprosthetic valve tissue and provides a low-cost, easy-to-manufacture, reproducible platform to optimize and analyze surface modification strategies. This model was manufactured from polyethylene glycol diacrylate (PEGDA) with collagen entrapped within the hydrogel matrix and incorporated a custom peptide coating, and optimized to mimic the local surface mechanics, surface chemistry, and fixation and detoxification processes characteristic of bioprosthetic valves.

2. Materials and methods

2.1. Materials

Glutaraldehyde (25 v/v% in water), eosin Y, 1-vinyl-2-pyrrolidinone and TEOA were purchased from Sigma Aldrich (St Louis, MO). The custom peptide, CRKRLQVQLSIRT, was purchased from American Peptide Company (Sunnyvale, CA). Valve leaflets were taken from porcine hearts purchased from Fisher Ham & Meat (Spring, TX). Bovine collagen type I (Vitrogen 100®) was purchased from Angiotech Biomaterials (Palo Alto, CA), then concentrated to 10 mg/mL in 0.024 N HCl.

2.2. Valve sample preparation

Porcine hearts were obtained from a local commercial abattoir, and leaflets were harvested from the aortic valve. A 5 mm biopsy punch was used to cut a specimen from the belly region of each leaflet. Fresh (unfixed) samples served as a baseline comparison in all studies, while experimental samples underwent fixation. Care was taken to identify the ventricularis and fibrosa sides of the leaflet prior to analysis by micropipette aspiration. Fixation of the leaflet tissue was carried out by incubating the tissue samples in a solution of 0.2% glutaraldehyde in PBS at room temperature for 72 h, as published previously [20–23].

2.3. Model surface synthesis

10 kDa PEGDA was synthesized in the lab as described previously [24,25]. Briefly, 10 kDa PEG (Sigma Aldrich) was dissolved in dichloromethane at a concentration of 1 M with trimethylamine and acryloyl chloride and reacted overnight. The resulting PEGDA was purified and acrylation verified by nuclear magnetic resonance (H^1 NMR). To create the BVSM, PEGDA was suspended at the appropriate concentration in a solution of 0.02 mM Eosin Y and 0.0655 M 1-vinyl-2-pyrrolidinone in Hank's balanced salt solution (HBS) with 3% triethanolamine (TEOA). This PEGDA solution was mixed with collagen type I in 0.024 N HCl at a ratio of 5:1 to produce a solution containing 0.5 mg/mL collagen type I. Collagen was incorporated into the hydrogel as a secondary model protein because it is the main component of the valve matrix that is targeted by glutaraldehyde during fixation of the tissue to prevent degradation *in vivo*. The PEGDA/collagen solution was then cast in a mold and crosslinked for 30 s under white light (~156 klux). The partially crosslinked gels were then allowed to swell overnight in PBS. It was also important that the BVSM be amenable to the amine-based surface modification chemistry to be implemented [14,21]. Therefore, a naturally-occurring amine-rich peptide, CRKRLQVQLSIRT, hereafter referred to as RKR, was chosen as a model peptide to mimic the protein content on the valve surface. This peptide is derived from laminin, a major component of the basement membrane present on the valve surface [26–28]. RKR was grafted to the BVSM surface at a concentration of 3 mM in a mixture of 0.01 mM Eosin Y and 0.03275 M 1-vinyl-2-pyrrolidinone in HBS with 3% TEOA. This mixture was added to the surface of each gel and crosslinked for 1.5 min under white light. Following peptide grafting, the resulting BVSM gels were vigorously rinsed 5× with PBS, then an additional 3 × 10 min in PBS to remove excess peptide before fixation or analysis. Samples were fixed using a 0.01% solution of glutaraldehyde in PBS for 24 h at room temperature. This change in fixation protocol from that used for tissue samples was made in response to preliminary data, as described in Appendix A.

2.4. Micropipette aspiration

Micropipette aspiration was used to measure surface mechanics, since it is well known that mechanics play an important role